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The Efflux of Amino Acids from the Olfactory Organ of the Spiny Lobster: Biochemical Measurements and Physiological Effects

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Abstract. The amino acids taurine and glycine are odorants that activate specific chemosensory cells in the olfactory sensilla (aesthetascs) of the spiny lobster, *Panulirus argus*. We show that the aesthetascs themselves contain large intracellular concentrations of taurine (≈2 m*M*) and glycine (≈85 m*M*); these concentrations are more than 10,000-fold greater than the response thresholds of the chemosensory cells. A net efflux of at least five amino acids occurs when the olfactory organ is immersed in amino acid-free seawater. With taurine and glycine, efflux continues until an apparent equilibrium is reached between the sensilla and the external medium; for taurine the equilibrium with seawater occurs at ≈12 to 28 n*M*, and for glycine at ≈100 to 500 n*M*. Aesthetascs may achieve these equilibria within 300 ms. Hence, even during the brief interval between consecutive flicks of the antennule, olfactory receptors are exposed to a background of odorants escaping from intracellular stores. Electrophysiological studies show that both the spontaneous and evoked activities of taurine-sensitive chemosensory cells are markedly affected by a taurine background simulating that measured in the efflux studies. Uptake systems may participate in establishing the equilibria between sensilla and seawater since (1) the net efflux of amino acids increases in sodium-free seawater; and (2) guanidinoethane sulfonate, a competitor for taurine uptake, selectively increases net taurine efflux. Effluxes from an olfactory organ may contribute noise to the chemosensory process; alternatively, background substances could contribute functionally by affecting membrane proteins.

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Abbreviations: ASW: artificial seawater; GES: guanidinoethane sulfonate; HPLC: high performance liquid chromatography; OPA: *ortho*-pthaldialdehyde.

Introduction

Olfactory sensilla on the antennules of the Florida spiny lobster, *Panulirus argus*, contain populations of chemoreceptor cells with differential specificities for taurine, glycine, and other amino acids (see review by Carr *et al.*, 1987). Physiological studies have revealed that taurine-sensitive cells have response thresholds of about 10^{-10} *M* (Fuzessery *et al.*, 1978; Ache *et al.*, 1988), with some cells being activated by taurine at concentrations as low as 10^{-12} *M* (Thompson and Ache, 1980). The response thresholds of the glycine-sensitive cells (≈10^{-6} *M*; Ache *et al.*, 1988) are generally higher than those of the taurine-sensitive cells. Chemoreceptors sensitive to exogenous amino acids are not unique to the spiny lobster. Indeed, chemoreceptor cells with selective sensitivities to specific amino acids also occur in several other crustaceans including the American lobster, *Homarus americanus* (Derby and Atema, 1982; Johnson and Atema, 1983), a crayfish, *Austropotamobius torrentium* (Hatt, 1984), a prawn, *Macrobrachium rosenbergii* (Derby and Harpaz, 1988), and a crab, *Carcinus maenas* (Schmidt and Gnatzy, 1989).

In addition to serving as exogenous chemoexcitants of crustaceans, amino acids such as taurine and glycine also occur intracellularly in very high concentrations (10^{-1} to 10^{-3} *M*), and contribute to osmotic regulatory processes (*e.g.*, Yancey *et al.*, 1982; Pierce, 1982). The antennular nerve of the spiny lobster, for example, contains taurine at a concentration of about 4 m*M* (see Results). Thus, this animal maintains an intracellular concentration of taurine that is more than a million-fold higher than the

response thresholds reported for its taurine-sensitive chemoreceptors. If the sensory cells themselves contain millimolar concentrations of taurine, then a small leakage from these cells into the receptor environment could generate a high background level of taurine that might negate the apparent utility of receptors with nanomolar sensitivities. The leakage of internal chemicals into the receptor environment has indeed been implicated in mammals, where chemostimulants injected into the blood stream activated olfactory (Maruniak *et al.*, 1983) and gustatory receptors (Bradley and Mistretta, 1971).

In the present study, we show that the olfactory sensilla of the spiny lobster contain high intracellular concentrations of free amino acids. Using an attached intact antennular preparation (Trapido-Rosenthal *et al.*, 1990), we show that there is a measurable efflux of at least five amino acids from the olfactory organ. Although the efflux of each amino acid appears to be regulated, the efflux of glycine is most pronounced and may produce glycine backgrounds of up to 5×10^{-7} *M* in the receptor environment. Finally, we demonstrate that the physiological responses of taurine-sensitive cells can be affected by a taurine background of about 10^{-8} *M* which occurs because of the efflux from intracellular pools.

Materials and Methods

Collection and maintenance of animals

Specimens of the Florida spiny lobster, *Panulirus argus*, were collected in the Florida Keys and maintained at the Whitney Laboratory in flowing seawater on a diet of fish, squid, and shrimp. Only adult intermolt animals were used.

Biochemical procedures

Amino acid analysis. Amino acids present in tissue samples or in aliquots of incubation media were derivatized with *ortho*-pthaldialdehyde (OPA) (Lindroth and Mopper, 1979). The fluorescent derivatives were separated by HPLC; an octadecylsilane column packed with 4 μm beads (Waters Nova-Pak C_{18}) and eluted with Buffers A and B was used as described by Manahan (1989). Buffer A consisted of 50 m*M* sodium acetate (pH 6.8), methanol, and tetrahydrofuran (80:19:1). Buffer B consisted of 50 m*M* sodium acetate and methanol (20:80). Derivatized amino acids were eluted from the column according to a step gradient procedure modified from Manahan (1989), so that at 1, 6, 11, and 16 min after sample injection, the percentage of Buffer B was increased from 0 to 25, 50, 75 and 100, respectively. The flow rate was 1.2 ml per min. Fluorescent derivatives were detected with a Bio-Rad Model 1700 fluorometer fitted with a 360 nm excitation filter and a 440 nm emission filter; peaks were integrated by means of a Waters Model 730 Data Module. Identification and quantitation of amino acid derivatives were performed by comparisons with standards.

Extraction of amino acids from tissues. Olfactory sensilla (= aesthetascs) were collected from lateral antennular filaments after blotting, rapid freezing in liquid nitrogen, and lyophilization. The sensilla were removed with fine-tipped forceps, and their numbers estimated by counting the antennular segments harvested. Antennular nerve sections, approximately 2 cm in length, were dissected from lateral antennular filaments at a position just proximal to the aesthetasc tuft. Dissections were performed in a bath of *P. argus* saline, and the tissue blotted, weighed, and frozen. Hemolymph samples (1 ml) were withdrawn at the base of a walking leg; a chilled syringe was used to minimize clotting. Samples were immediately centrifuged ($12{,}000 \times g$) to remove cellular material, and the supernatant frozen.

Free amino acids in tissue samples were extracted by homogenization in a solution of 80% methanol/20% sodium acetate (50 m*M*, pH 6.8) followed by centrifugation ($16{,}000 \times g$). The supernatants were transferred to clean tubes, evaporated to dryness, then redissolved in 50 m*M* sodium acetate for reaction with OPA as described above.

Net efflux of amino acids from the aesthetasc sensilla of attached intact antennules. Lobsters were removed from the water and immobilized on racks as described previously (Trapido-Rosenthal *et al.*, 1990). The distal portions of the intact lateral antennular filaments were placed in vials containing 3.5 to 4.5 ml of artificial seawater (ASW; see Gleeson *et al.*, 1989) which was vigorously agitated by using magnetic stirring bars. At selected times, samples of the ASW (= incubation medium) were removed from the vials for amino acid analysis. The above procedure ensured that the only part of the lobster contacting the incubation medium was the antennular filament; all references to the use of intact antennules are references to this procedure.

In one experiment, analyses were performed on amino acids released into ASW by both aesthetasc-bearing and aesthetasc-free sections of antennular cuticle. These isolated sections were prepared as described in Trapido-Rosenthal *et al.* (1987).

In experiments to investigate the effect of low concentrations of sodium on amino acid efflux from the aesthetascs, intact antennules were subjected to three sequential, 10-min incubations. In the first incubation, the antennule was immersed in ASW; in the second incubation the antennule was immersed in artificial seawater in which the sodium chloride had been replaced with equimolar choline chloride. The third incubation was again performed in ASW. The effect of the taurine-uptake competitor, guanidinoethane sulfonate (GES), on amino acid efflux was examined according to a similar protocol

except that the second of the three incubations contained 1 μM GES in ASW.

Electrophysiological procedures

The responses of single cells stimulated by taurine were recorded extracellularly from the isolated perfused lateral filament of the antennule. The olfactometer and recording procedures have been described in detail previously (Gleeson and Ache, 1985). Action potentials (impulses) from single cells were discerned via an amplitude/time window discriminator, and the time intervals between impulses analyzed with a microprocessor. In this report, cell responses are quantified in two ways: (1) the total number of impulses occurring within a 5- or 10-s period following onset of the response; and (2) maximum frequency, defined as the mean instantaneous frequency determined for the four shortest intervals between successive impulses.

Effects of background taurine. Cells stimulated by taurine were identified by introducing a 10-μM search stimulus into the carrier stream of ASW that continuously flowed past the olfactory sensilla at a rate of 3 ml/min. Once a taurine-sensitive cell had been identified, the dose-response function was determined in the presence and absence of an imposed background of 10 nM taurine. This background simulated a representative concentration present in the receptor environment as calculated from our measurements of the efflux of endogenous taurine (*vide infra*). The effect of the background on the dose-response function was determined by applying an ascending series of taurine concentrations; each concentration was tested with and without background before the next higher concentration in the series was applied. For tests in the presence of background, the carrier stream of ASW contained 10 nM taurine which flowed through the olfactometer for 2 min before and during the introduction of a test stimulus. The response to each concentration was monitored following the injection of 190 μl into the carrier flow of ASW. With this volume, the concentration of taurine in the olfactometer reached the injected level within 1 s and began to decline after a 2-s plateau period (Zimmer-Faust *et al.*, 1989). Following the presentation of each stimulus, the preparation was flushed with ASW for 2 min.

To determine whether taurine-sensitive cells are affected by taurine circulating in the hemolymph, cell responses were examined while the antennule was perfused with *P. argus* saline containing various concentrations of this amino acid. For these experiments, the responses to exogenous taurine injected into the carrier flow of ASW were compared in the presence and absence of taurine in the perfusion medium. For tests with taurine in the perfusion saline, cell responses were examined at 5-min intervals for up to 25 min.

Stimulus preparation. Taurine stock solutions (1 mM) were prepared in ASW, the pH adjusted to 7.8, and aliquots stored at −70°C. At the beginning of each experiment, test stimuli were prepared by serially diluting the stock solution with ASW. For stimuli presented in the presence of a taurine background, stock solutions were serially diluted in ASW containing 10 nM taurine.

Reagents

Amino acids and the fluorescent derivatizing reagent *ortho*-pthaldialdehyde (OPA) were purchased from Sigma Chemical Company. Guanidinoethane sulfonate (GES) was generously provided by Dr. Ryan Huxtable. Reagent-grade salts were from Fisher Scientific Company, as were HPLC-grade methanol and tetrahydrofuran.

Results

Amino acid content of the aesthetasc sensilla

Aesthetascs on the lateral filament of the antennule contain high intracellular concentrations of several free amino acids. A chromatogram of amino acids extracted from aesthetascs appears in Figure 1; intracellular concentrations of five of these amino acids are shown in Figure 2. Glycine occurs in the sensilla at a concentration of 85.2 ± 2.4 mM and is the predominant OPA-derivatizable amino acid in the intracellular pool. Taurine is a far more minor constituent, occurring at a concentration of 2.1 ± 0.7 mM.

The composition of free amino acids in the aesthetascs differs markedly from that of both the antennular nerve and the hemolymph (Fig. 2). For example, glycine is the major component within both the aesthetascs and hemolymph, but is superseded by aspartate in antennular nerve; taurine, which is a relatively minor constituent in the aesthetascs and antennular nerve, occurs at a mid-range concentration within the hemolymph. In addition to these differences in relative concentrations between tissues, the absolute concentrations of the amino acids in hemolymph are considerably lower than those within the aesthetascs and antennular nerve.

Amino acid efflux from the aesthetasc sensilla

When intact antennules are placed into amino acid-free ASW, a net efflux of amino acids occurs (Fig. 3). During 10-min incubations, glycine efflux ranged from 51.5 to 299.9 fmoles per sensillum, whereas taurine efflux ranged from below the limits of detection by OPA to 29.5 fmoles per sensillum. We assumed that the aesthetascs are the major sites of amino acid efflux based upon the following considerations. (1) The aesthetascs on each antennule contain approximately 12.5 cm^2 of dendritic membrane which is separated from the external seawater

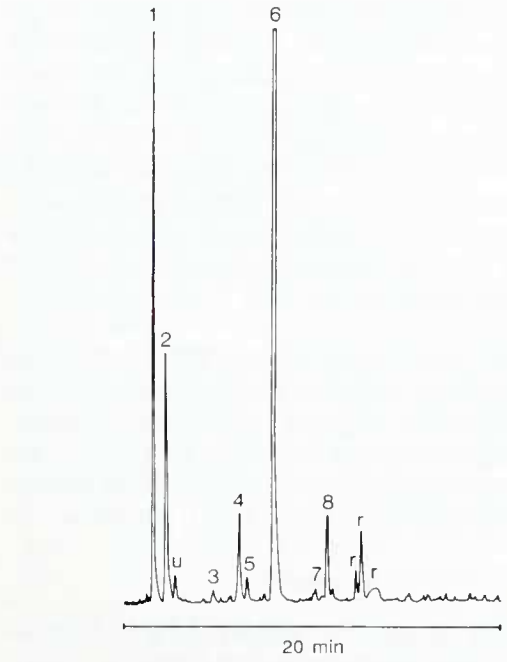

Figure 1. HPLC chromatogram of free amino acids extracted from 90 aesthetasc sensilla from a single antennule. 1 = aspartic acid (92 pmoles); 2 = glutamic acid (41 pmoles); 3 = asparagine (7 pmoles); 4 = serine (18 pmoles); 5 = histidine (16 pmoles); 6 = glycine (370 pmoles); 7 = taurine (4 pmoles); 8 = alanine (17 pmoles); r = reagent peaks; u = unknown.

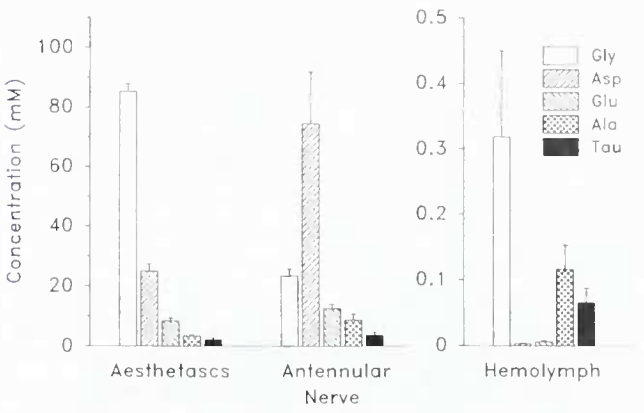

Figure 2. Mean concentrations (+SEM) of selected free amino acids in aesthetasc sensilla, antennular nerve and hemolymph of the spiny lobster. Intracellular concentrations for aesthetascs are based on the assumption that cells occupy 20% of the 250 pl volume of each sensillum (calculated from Grünert and Ache, 1988). For determining antennular nerve concentrations, tissue wet weights were converted to volume assuming a specific gravity of unity. Tissues from six animals were used in these determinations, except that the aesthetasc sensilla from the antennules of four animals were used. Gly = glycine; Asp = aspartic acid; Glu = glutamic acid; Ala = alanine; Tau = taurine.

environment by only a thin layer of permeable cuticle (surface area calculated from the data of Grünert and Ache, 1988). This surface area far exceeds the cell-membrane area exposed by other cuticular structures on the lateral filament of the antennule. (2) The concentration profiles of amino acids present within the aesthetascs and in the efflux from the antennule are markedly similar (Inset, Fig. 3). (3) The amino acid effluxes from aesthetasc-bearing and aesthetasc-free sections of antennular cuticle were compared, and the efflux from the former exceeded the latter by 25-fold. Sensilla-associated microorganisms may have made positive or negative contributions to the measured effluxes, but neither light nor electron microscopy have revealed extensive populations of such organisms (R. A. Gleeson, pers. comm.).

Amino acid transport in invertebrates, like that of vertebrates, is a Na^+-dependent process (*e.g.*, Gunn, 1980; Stevens *et al.*, 1984; Wright and Pajor, 1989). The Na^+-dependence of these transport systems is presumed to represent a carrier-mediated co-transport of Na^+ in which the energy is provided by the transmembrane Na^+ gradient. Incubation of antennules in Na^+-free ASW caused a reversible increase in the net efflux of amino acids (Fig. 4). The enhancement of efflux was greater than twofold for each amino acid measured and was restored to control levels when the antennules were returned to Na^+-containing ASW. This enhancement was significant for each of the amino acids with the exception of taurine [Repeated Measures ANOVA coupled with a Planned Comparisons Test (CSS, StatSoft): $P < 0.05$, n = 3]. The data for taurine did, however, approach significance ($P = 0.06$).

The net efflux of taurine from antennules showed about a five-fold enhancement in the presence of 1 μM GES, a specific competitor for taurine and other substances internalized by β-amino acid transport systems (Huxtable *et al.*, 1979; Quesada *et al.*, 1984) (Fig. 5). The enhancement of taurine efflux was significant [Repeated Measures

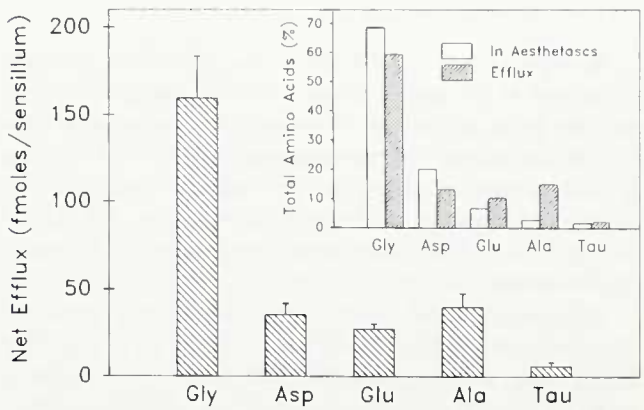

Figure 3. Net efflux of amino acids from intact antennules during 10-min incubations in ASW. Values are the means + SEM for 14 animals. Inset compares the profiles of amino acids in the aesthetascs (as shown in Fig. 2) with those released into the ASW. Amino acid abbreviations as in Figure 2.

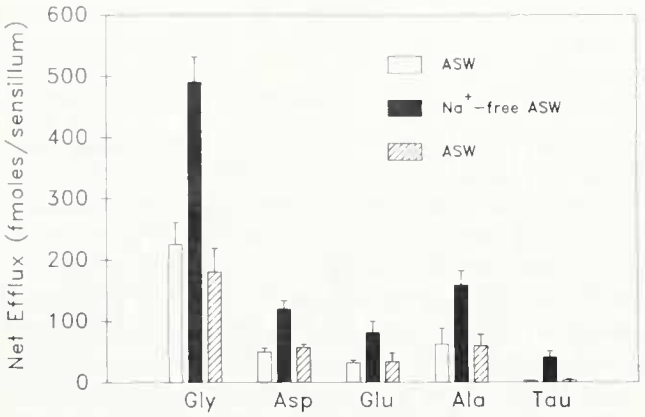

Figure 4. Efflux of amino acids from intact antennules incubated sequentially in ASW (open bars), Na^+-free ASW (solid bars), and ASW (hatched bars). In Na^+-free ASW, NaCl was replaced with equimolar choline chloride. Incubations were for 10 min. Values are the means + SEM of three experiments. Amino acid abbreviations as in Figure 2.

ANOVA coupled with a Planned Comparison Test (CSS, StatSoft): $P < 0.05$, n = 3] and reversible. GES did not significantly affect the efflux of the other amino acids examined.

The time course for the appearance of glycine and taurine in the incubation medium was determined for the antennules of three different animals (Fig. 6). Although considerable inter-animal variability existed, in each case and for both amino acids, the extracellular concentration increased until an apparent equilibrium concentration was attained. The equilibrium concentrations ranged from 100 to 500 n*M* for glycine (Fig. 6A) and from 12 to 28 n*M* for taurine (Fig. 6B). The occurrence of a rapid efflux to an equilibrium concentration was typically observed for other amino acids as well.

Physiological effects of a taurine background

In the presence of a 10 n*M* background concentration of taurine in the carrier stream of ASW, the spontaneous activity in all of the taurine-sensitive cells examined obviously increased. This increase was significant; following a 2-min exposure to the taurine background, the mean number of impulses per second was 4.26 ± 0.28 (±SEM) *versus* 0.88 ± 0.08 in the absence of taurine (Wilcoxon Signed Rank Test: $P < 0.01$, n = 9).

The dose-response function for injected (= exogenous) taurine in the presence and absence of the 10 n*M* taurine background is shown in Figure 7. Expressing the response in terms of either maximum frequency or impulses per 5 s reveals a dose-dependent increase in activity which appears to attain a maximum level between 3.3 and 10 μM taurine. In the presence of the taurine background, there is an apparent downward shift in the dose-response function. This shift is highly significant as revealed by the intercept differences for the linear regions (*i.e.*, between 0.01 and 3.3 μM taurine) of the maximum frequency curves [Random Coefficient Regression Analysis with intercepts compared using a Wilcoxon Signed Rank Test: $P = 0.004$, n = 9]. A paired comparison of the maximum responses (*i.e.*, responses to 10 and 33 μM taurine) for cells in the presence and absence of background taurine also yielded a significant difference [Wilcoxon Signed Rank Test: $P = 0.03$ (maximum frequency data), $P < 0.001$ (impulses per 5 s data), n = 15].

The responses of taurine-sensitive cells to exogenous taurine stimuli were unaffected by the presence of taurine in the perfusion saline (Fig. 8). For the six cells examined, the mean responses to test stimuli in the presence and absence of taurine in the perfusion medium were virtually identical (Wilcoxon Signed Rank Test: $P = 0.438$, n = 6).

Discussion

Cells within the aesthetasc sensilla of the spiny lobster contain the amino acids glycine and taurine at concentrations of about 85 and 2 m*M*, respectively. Following immersion of the intact lateral antennular filament in amino acid-free seawater, a net efflux of glycine and taurine from the sensilla occurs until an apparent equilibrium is reached with the external medium; for glycine the equilibrium concentration in the seawater is about 100 to 500 n*M*, and for taurine it is approximately 12 to 28 n*M* (Fig. 6). The establishment of these equilibria indicates that aesthetascs can maintain intracellular glycine and taurine at concentrations that are more than 100,000-fold greater than those in the external medium. However, the existence of apparent limits on the ratio of intracellular to extracellular concentrations suggests that, even in seawater free

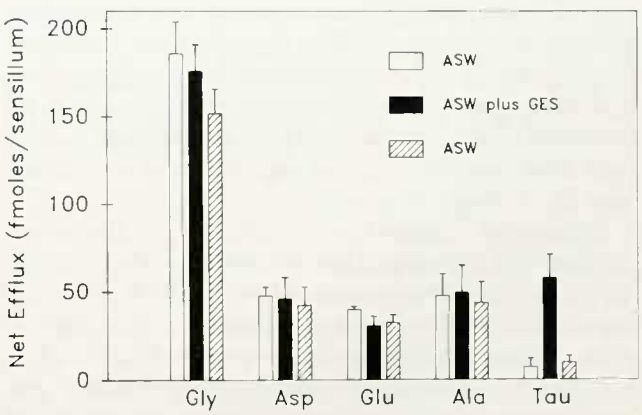

Figure 5. Efflux of amino acids from intact antennules incubated sequentially in ASW (open bars), ASW containing 1 μM GES (solid bars), and ASW (hatched bars). Incubations were for 10 min. Values are the means + SEM of three experiments. Amino acid abbreviations as in Figure 2.

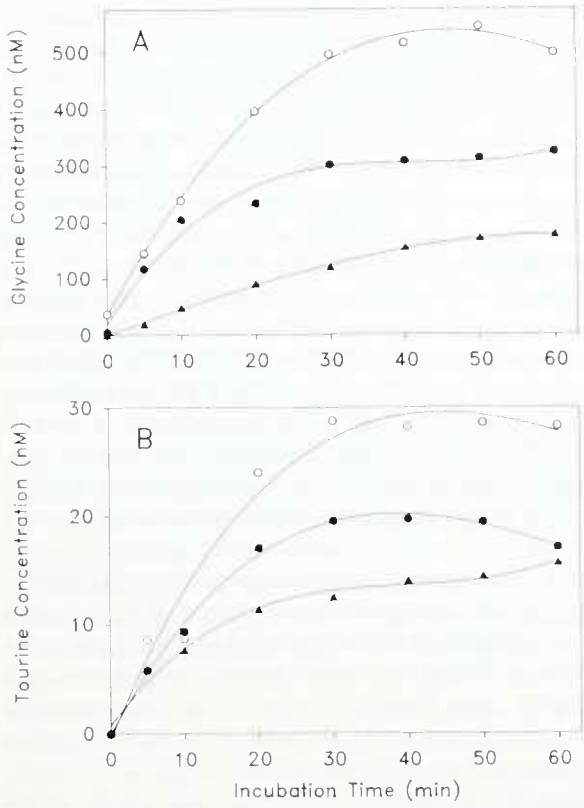

Figure 6. The time course of efflux for glycine (A) and taurine (B) from intact antennules of three animals. At each time point, 100-μl aliquots were removed from the incubation vials and the amino acid concentrations in the incubation media were determined. Apparent equilibrium concentrations were attained in the 4.5-ml incubation volumes within 30 to 40 min.

of exogenous glycine or taurine, sensillar receptors will be exposed to background ("noise") levels of these amino acids because of their efflux from intracellular pools.

The effluxes of glycine and taurine from the olfactory organ create, at equilibrium, background levels that correlate quite well with those occurring in natural seawater. Glycine is frequently present in seawater at levels approaching 100 n*M* or greater (*e.g.*, Garrasi *et al.*, 1979; Braven *et al.*, 1984; Siebers and Winkler, 1984), whereas taurine is often not detected and seldom exceeds 10 n*M* (Mopper and Lindroth, 1982; Wright and Secomb, 1986). The existence of a low background (*i.e.*, low noise) level of taurine in seawater, plus the occurrence of only a slight efflux from the olfactory organ, suggests that taurine leaking from a prey organism would be more readily detected than glycine. For taurine, an effective signal-to-noise ratio could exist at exogenous concentrations above approximately 10 n*M*; whereas for glycine, good signal-to-noise ratios would require concentrations greater than about 100 n*M*. Indeed, the contrasts between the low (nanomolar) thresholds of taurine-sensitive cells (Fuzessery *et al.*, 1978; Ache *et al.*, 1988), and the apparently higher (micromolar) thresholds of glycine-sensitive cells (Ache *et al.*, 1988), may be expressions of receptor adaptations to the exigencies of different background concentrations.

The lobster obtains discontinuous samples of its chemical environment by periodically flicking its antennules in a manner that rapidly exchanges water trapped between the densely arranged aesthetasc sensilla (Price and Ache, 1977; Schmitt and Ache, 1979; Moore and Atema, 1988; R. A. Gleeson, pers. comm.). The time interval between successive flicks (interflick interval) can vary from about 500 ms to over 30 s (R. A. Gleeson, pers. comm.). During this interflick period, seawater trapped between the aesthetascs forms a large boundary layer within which odorant movement is essentially restricted to molecular diffusion (Schmitt and Ache, 1979; Moore and Atema, 1988). As a consequence, chemoreceptors within the aesthetascs are primarily exposed to whatever odorants are captured during the preceding flick, with the actual concentrations at the receptors being dependent upon the rates of odorant diffusion between the aesthetascs and the

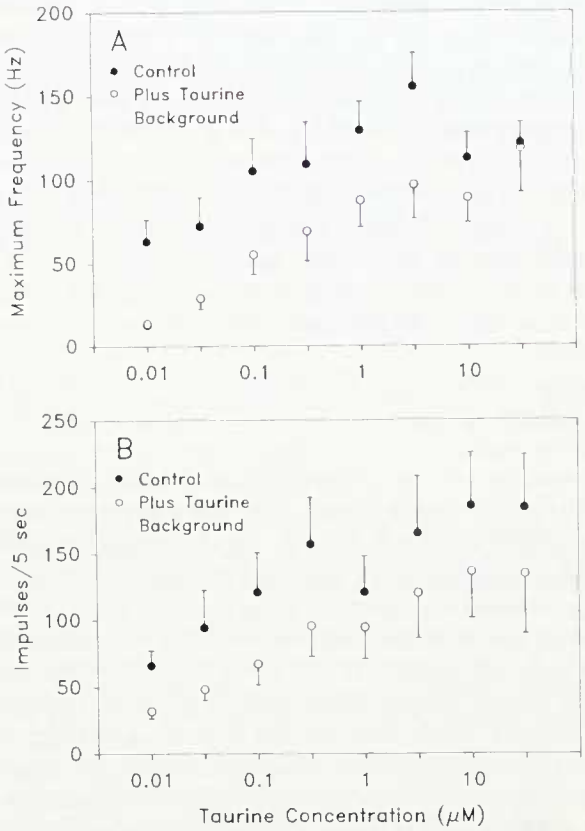

Figure 7. Dose-response functions for taurine-sensitive cells in the presence and absence of a 10 n*M* taurine background. Response magnitude is expressed in terms of maximum frequency (A) and total number of impulses during the first 5 s of the response (B). Points are the means ± SEM for nine cells.

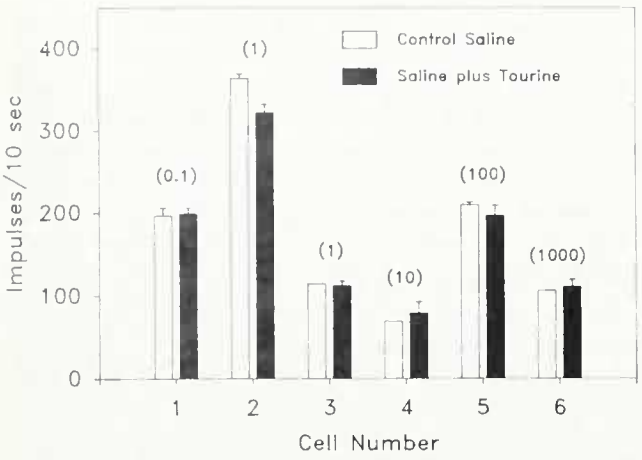

Figure 8. Mean responses (+SEM) of taurine-sensitive cells in the presence and absence of taurine in the perfusion saline. The micromolar concentrations of taurine in the perfusion medium are indicated in parentheses. For each cell, a test-stimulus concentration close to the EC_{50} was presented via the carrier stream of ASW.

boundary layer of seawater. This boundary layer can also limit the rate of amino acid efflux from aesthetascs by acting as a buffer between the sensilla and seawater outside the aesthetasc tuft. If it is assumed that a major fraction of the glycine and taurine leaking from the cells of an aesthetasc during the interflick interval remains within the sensillar lymph (volume $\approx$ 200 pl), then only about 300 ms would be required for the sensillum to attain the equilibrium concentrations measured in the current study (Fig. 9). Hence, during a considerable portion of each interflick interval, the sensillar receptors are probably exposed to backgrounds ranging from low nanomolar in the case of taurine, to as high as 0.5 micromolar in the case of glycine.

When sensilla are immersed in seawater, the efflux of glycine and taurine from intracellular stores does not continue unabated until the concentration in the sensilla and medium are equal. Rather, at equilibrium the intracellular concentration is about 100,000-fold greater than the medium. Uptake systems in the sensilla are the most plausible mechanisms for regulating the amino acid efflux. The hypothesis that uptake systems might control the net efflux or loss of intracellular amino acids was proposed by Wright and Secomb (1986) based on studies with the gills of marine mussels. These workers noted that mussel gills contain intracellular taurine at a concentration of about 60 m*M*, and that a net efflux occurred into seawater. They demonstrated the existence of a taurine uptake system and showed that it was able to recapture up to 30% of the taurine escaping from the gills. They then proposed that this re-uptake conserves energy and contributes to maintaining the high intracellular concentrations of taurine (Wright and Secomb, 1986; Wright, 1987). Our study on amino acid efflux from the lobster olfactory organ reveals the following parallels with these findings from molluscan gills: (1) immersion in amino acid-free seawater results in a net efflux of amino acids that continues until an apparent equilibrium is established with the external medium (Fig. 6); (2) the net efflux of amino acids increases in Na^+-free seawater (Fig. 4); and (3) a selective competitor of taurine uptake increases the net taurine efflux (Fig. 5) (Wright and Secomb, 1984, 1986; Wright *et al.*, 1989). Regarding the olfactory organ of the lobster, we already know that uptake systems for taurine and other amino acids are present in the olfactory sensilla (Gleeson *et al.*, 1987; Trapido-Rosenthal *et al.*, 1988). However, the kinetics of uptake exhibited by the excised sensilla used in these earlier studies are not compatible with maintaining the equilibrium concentrations measured in the current study. The intact antennular preparation should now be employed to re-examine the kinetics of uptake.

In the American lobster, *Homarus americanus*, the adaptation of NH_4-sensitive chemosensory cells to increased background levels of NH_4 was studied in detail by Borroni and Atema (1988). For each imposed background, adaptation of these receptor cells included: (1) a re-setting of the response threshold to an NH_4 concentration greater than background; and (2) a concomitant, parallel rightward shift in the stimulus-response function (*ibid.*). In the present study, the exposure of taurine-sensitive cells of the spiny lobster, *P. argus*, to a background level of taurine yielded results having both similarities and differences to those described for the NH_4-cells of *H. americanus*. Unlike

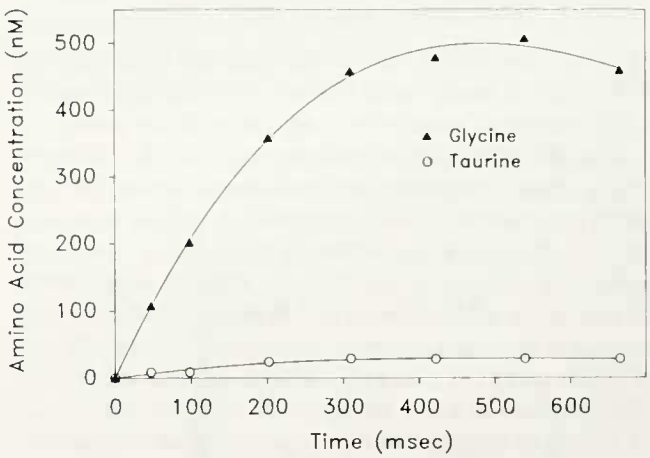

Figure 9. Calculated concentrations for glycine and taurine in the sensillar lymph of a single sensillum from the animal represented by open circles in Figure 6. Assumptions used were as follows. (1) At t_0, the antennule is flicked and the 200-pl extracellular volume within the sensillum immediately equilibrates with the amino acid-free seawater. (2) During the subsequent interflick interval, all amino acids released from the cells of the sensillum are retained in the 200-pl volume of sensillar lymph. Under these conditions, both glycine and taurine attain their equilibrium concentrations in the lymph within 300 ms.

the responses of NH_4-cells to NH_4 backgrounds, taurine-sensitive cells did not exhibit complete adaptation to the taurine background examined. Instead, after an initial phasic response when the background was first introduced, these cells reached a new tonic level of activity that was significantly greater than their spontaneous activity in ASW. As in NH_4-cells, adaptation (albeit partial) was indicated by a rightward, or seemingly downward, shift in the dose-response function. In the taurine-sensitive cells, this shift included an apparent reduction in the maximum response; this effect might be considered functionally equivalent to either a generalized reduction in the efficacy of taurine, or to an inactivation of some proportion of the receptor population.

In the current study, the responses of taurine-sensitive chemosensory cells in the antennule were not affected when taurine concentrations as high as 1 m*M* were presented internally via the perfusion saline (Fig. 8). These results imply that a functional barrier, at least for taurine, separates the hemolymph and the sensillar lymph. These results contrast with findings in mammals where certain chemostimulants injected into the blood stream were found to stimulate olfactory (Maruniak *et al.*, 1983) and gustatory receptors (Bradley and Mistretta, 1971).

A high intracellular concentration of low molecular weight organic substances is a characteristic feature of organisms subjected to water stresses, including high or fluctuating salinity (Yancey *et al.*, 1982). These organic substances together with inorganic ions represent the major osmotically active solutes (osmolytes) present within all cells. Prominent among these compounds are certain amino acids including taurine, glutamate, alanine, glycine, proline, and aspartate (Clark, 1985). The similarities between species in the chemical properties of organic osmolytes are remarkable; these properties parallel those of cations and anions in the Hofmeister series that favor compatibility with protein structure and function. Indeed, these intracellular substances may be important in offsetting the destabilizing or perturbing effects that high neutral salt concentrations have on macromolecules (Yancey *et al.*, 1982). By analogy with the intracellular effects described above, the efflux of certain amino acids (*e.g.*, glycine) and other osmolytes into the receptor environment within olfactory sensilla may play a general role in stabilizing the extracellular domains of various membrane proteins associated with the sensory dendrites. The efflux of such compounds could be important in maintaining the structure and function of receptors, channel proteins, transporters and ecto-enzymes that would otherwise be directly exposed to seawater. Indeed, certain of these substances may specifically modulate the activity of some membrane proteins. For example, extracellular glycine occurring in synaptic clefts binds to specific sites on the NMDA-glutamate receptor-channel complex and contributes significantly to receptor activation and channel function (*e.g.*, Kessler *et al.*, 1989; Thomson, 1989). Organic osmolytes in the extracellular lymph of olfactory sensilla may play similar regulatory roles in chemosensory processes.

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